FIG. 1a.

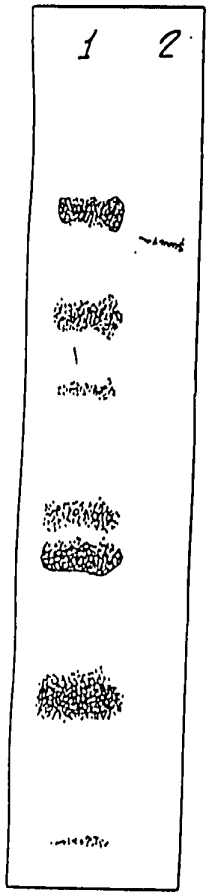


FIG. 1b.

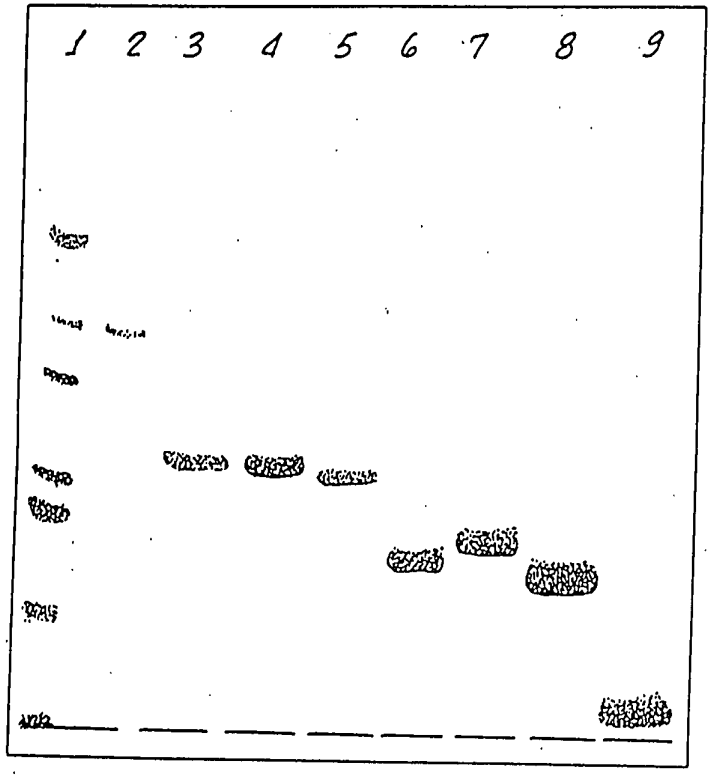


FIG. 1c.

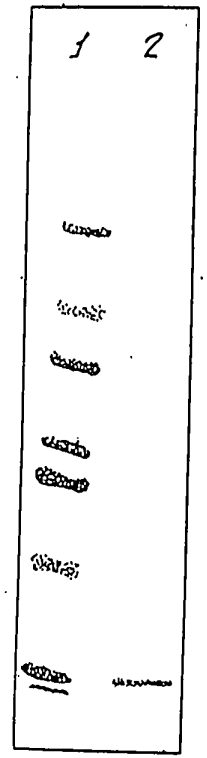


FIG. 1d.

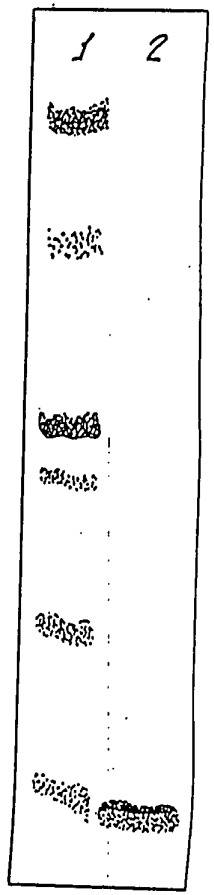


FIG. 2.

PURIFIED EXTRACELLULAR PROTEINS STUDIED	
APPARENT MW BY SDS-PAGE (KD)	N TERMINAL 5 AMINO ACIDS
110	NSKSV
80	TDRVS
*71	ARAVG
58	TEKTP
45	DPEPA
*32A	FSRPG
32B	FSRPG
*30	FSRPG
24	APYEN
23.5	APKTY
*23	AETYL
*16	AYPIT
14	ADPRL
12	FDTRL

FIG. 3.

EXTENDED N-TERMINAL SEQUENCE OF 30/32 KD COMPLEX OF M. TUBERCULOSIS EXTRACELLULAR PROTEINS

	1									10										20
30	F	S	R	P	G	L	P	V	E	Y	L	Q	V	P	S	P	S	M	G	R
32A	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
32B	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	A	–	–	–	–
	21									30										40
30	D	I	K	V	Q	F	Q	S	G	G	N	N	S	P	A	V	Y	L	L	D
32A	–	–	–	–	–	–	–	–	–	A	–	–	–	–	L	–	–	–	–	
32B	–	–																		

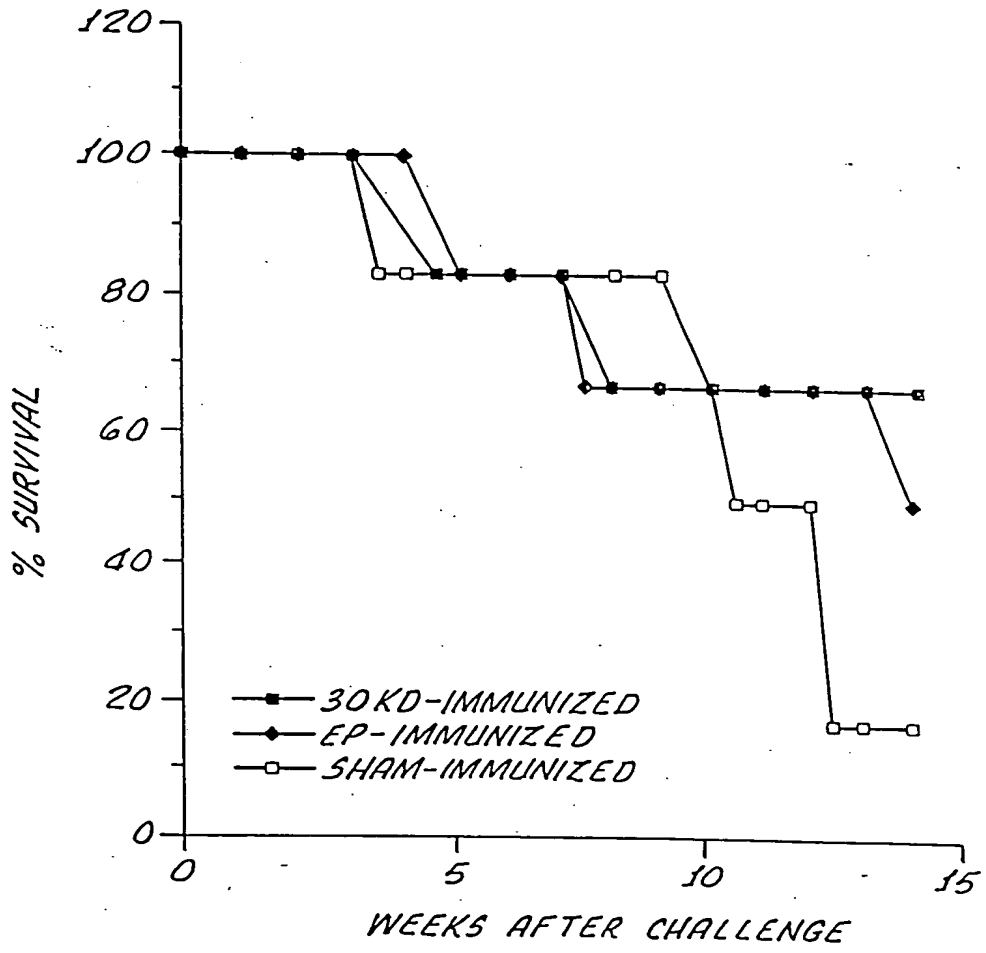
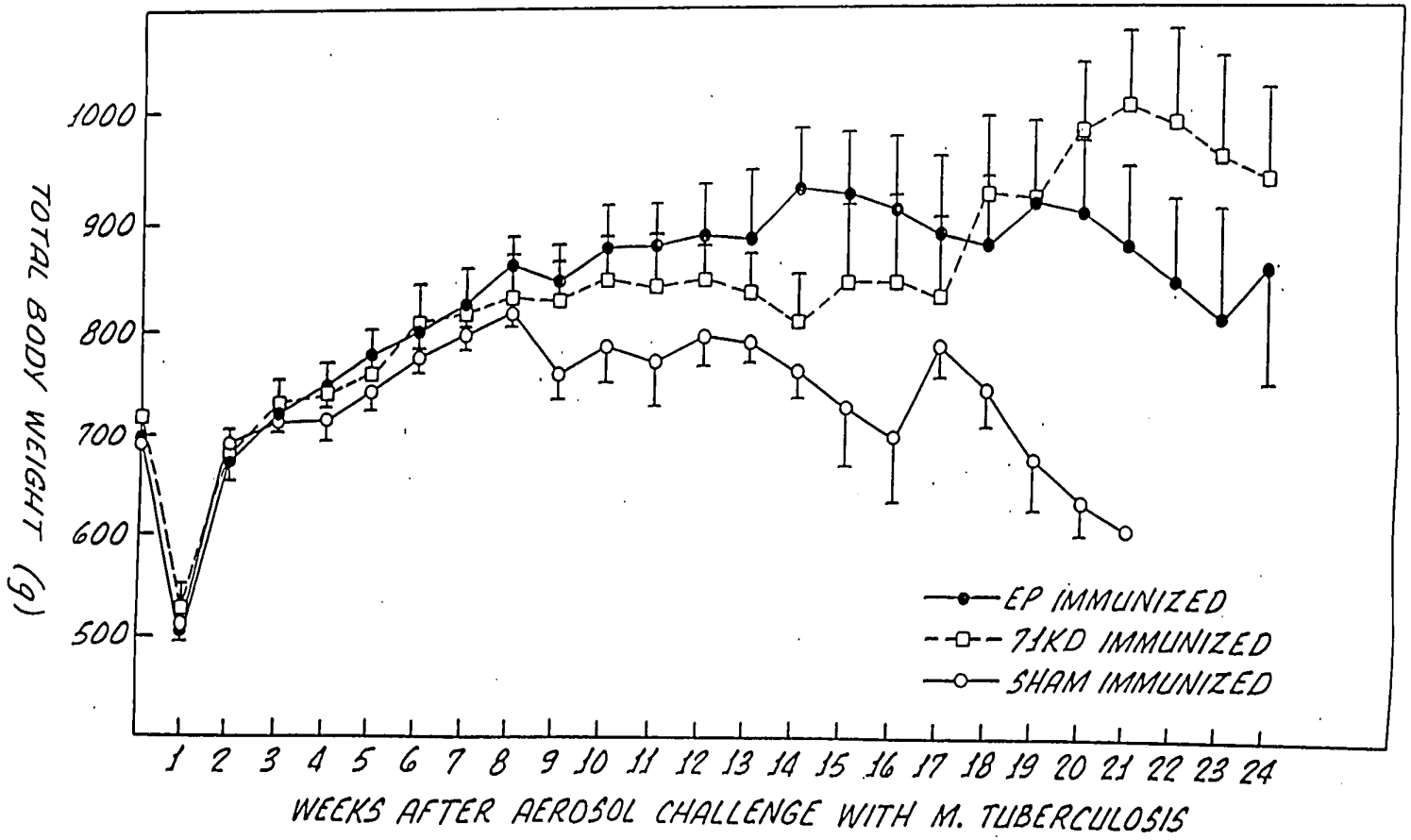

120
100
80
60
40
20
0
% SURVIVAL
0
5
10
15
WEEKS AFTER CHALLENGE
30KD-IMMUNIZED
EP-IMMUNIZED
SHAM-IMMUNIZED

FIG. 4.

FIG. 5.


TOTAL BODY WEIGHT (g)
1000
900
800
700
600
500
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
WEEKS AFTER AEROSOL CHALLENGE WITH M. TUBERCULOSIS
EP IMMUNIZED
71KD IMMUNIZED
SHAM IMMUNIZED

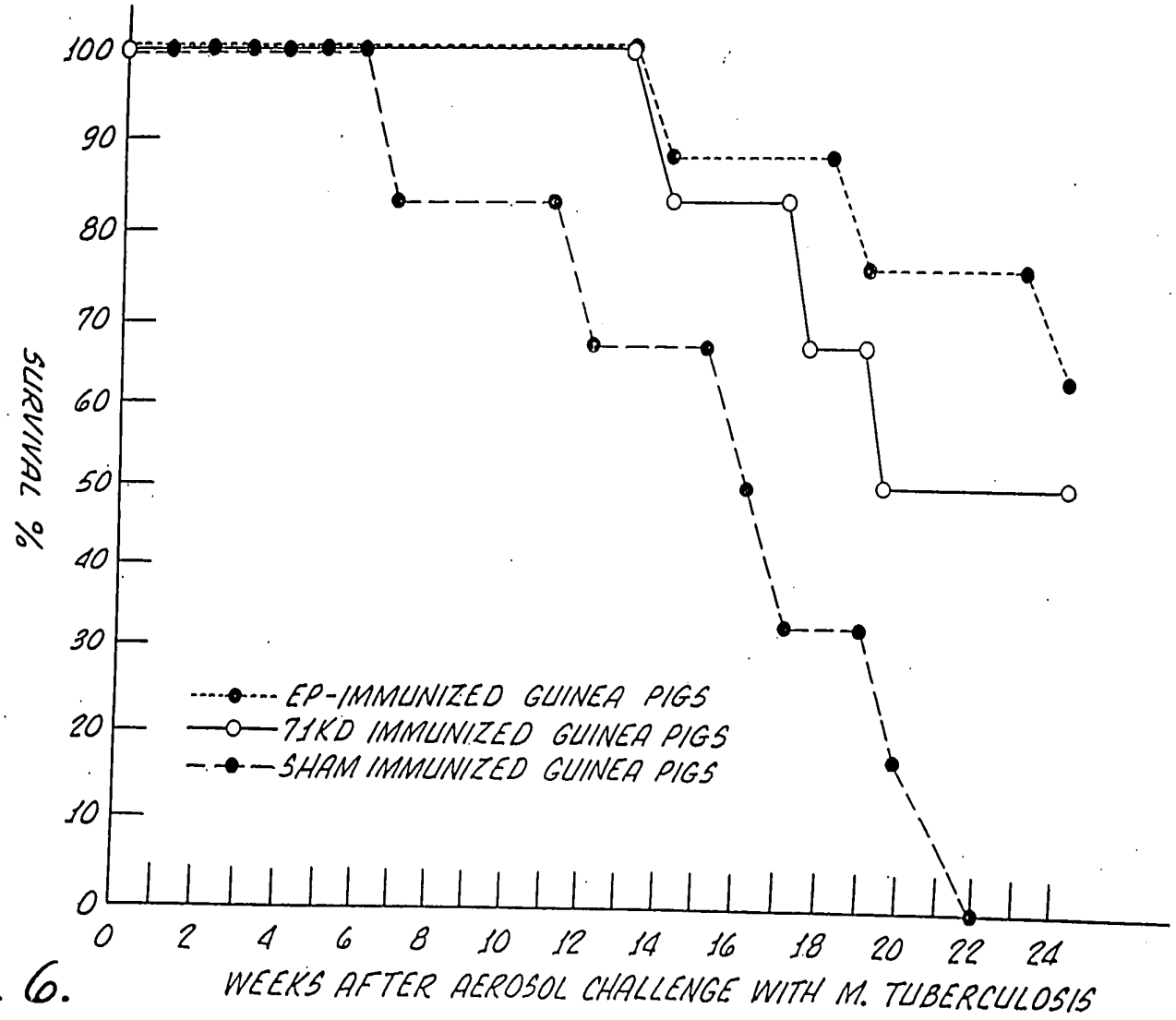

100
90
80
70
60
50
40
30
20
10
0
% SURVIVAL
EP-IMMUNIZED GUINEA PIGS
71KD IMMUNIZED GUINEA PIGS
SHAM IMMUNIZED GUINEA PIGS
0
2
4
6
8
10
12
14
16
18
20
22
24
WEEKS AFTER AEROSOL CHALLENGE WITH M. TUBERCULOSIS

FIG. 6.

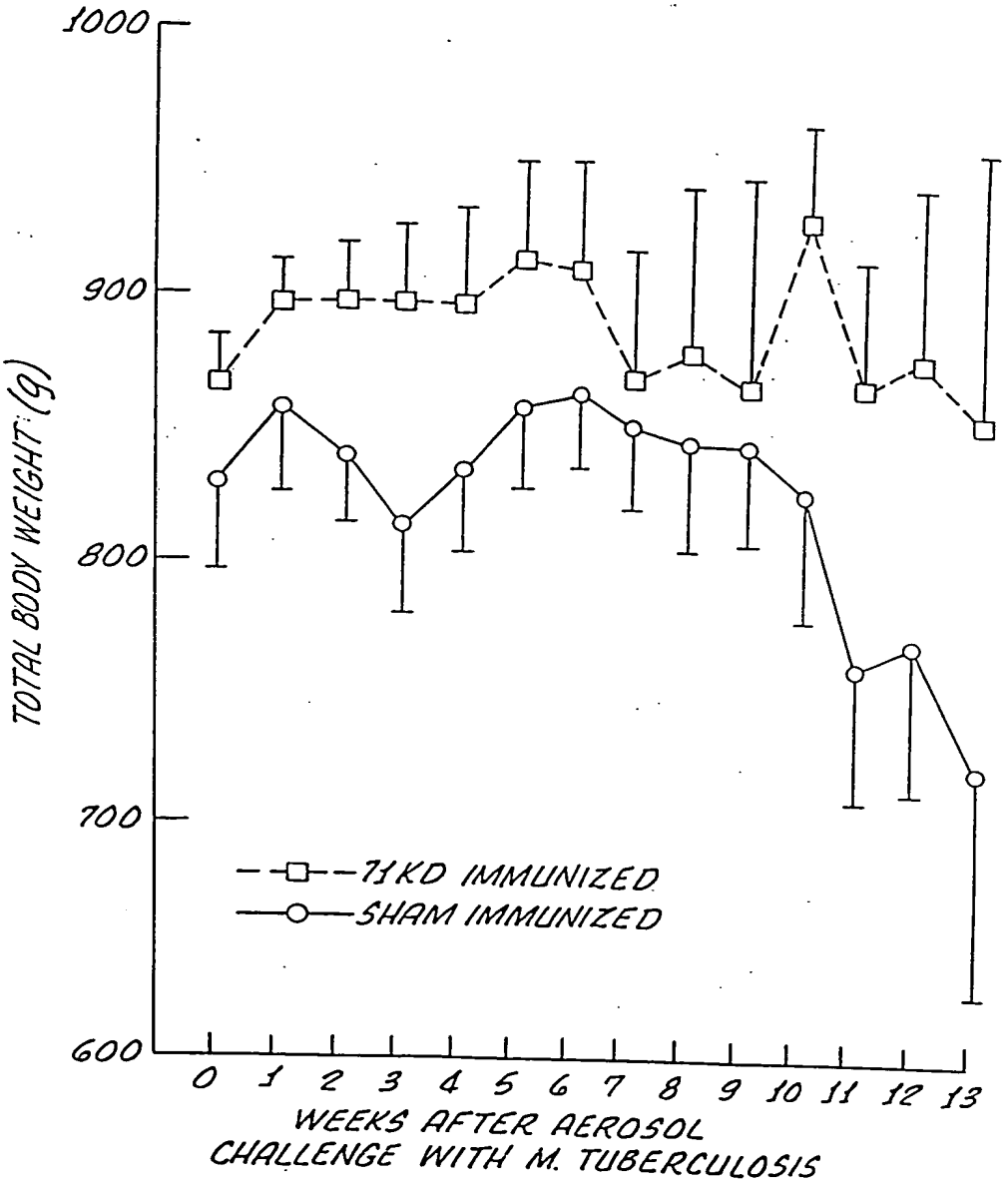

1000
900
800
700
600
TOTAL BODY WEIGHT (g)
71KD IMMUNIZED
SHAM IMMUNIZED
0 1 2 3 4 5 6 7 8 9 10 11 12 13
WEEKS AFTER AEROSOL
CHALLENGE WITH M. TUBERCULOSIS

FIG. 7.

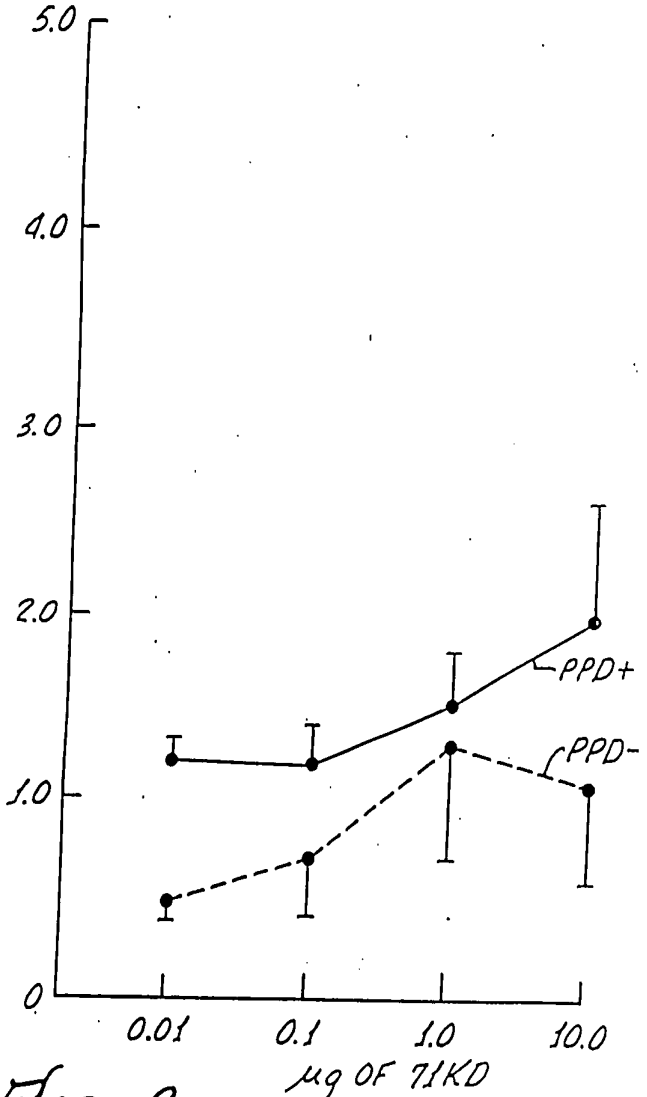

5.0
4.0
3.0
2.0
1.0
0
PPD+
PPD-
0.01
0.1
1.0
10.0
μg OF 71KD

FIG. 8a.

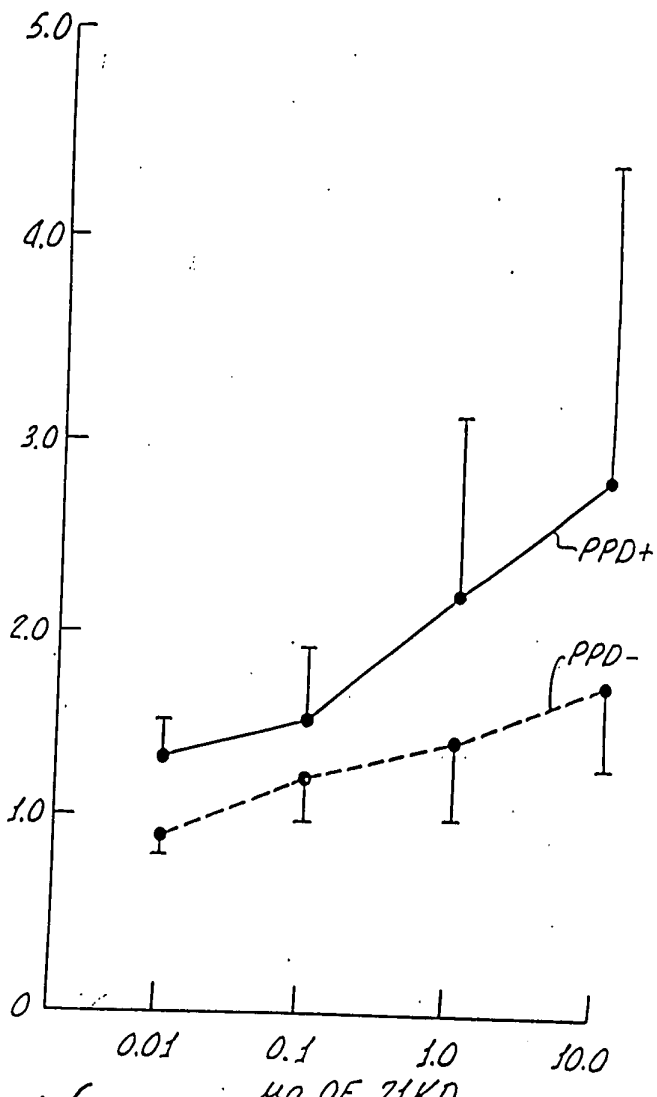

5.0
4.0
3.0
2.0
1.0
0
PPD+
PPD-
0.01
0.1
1.0
10.0
μg OF 71KD

FIG 8b

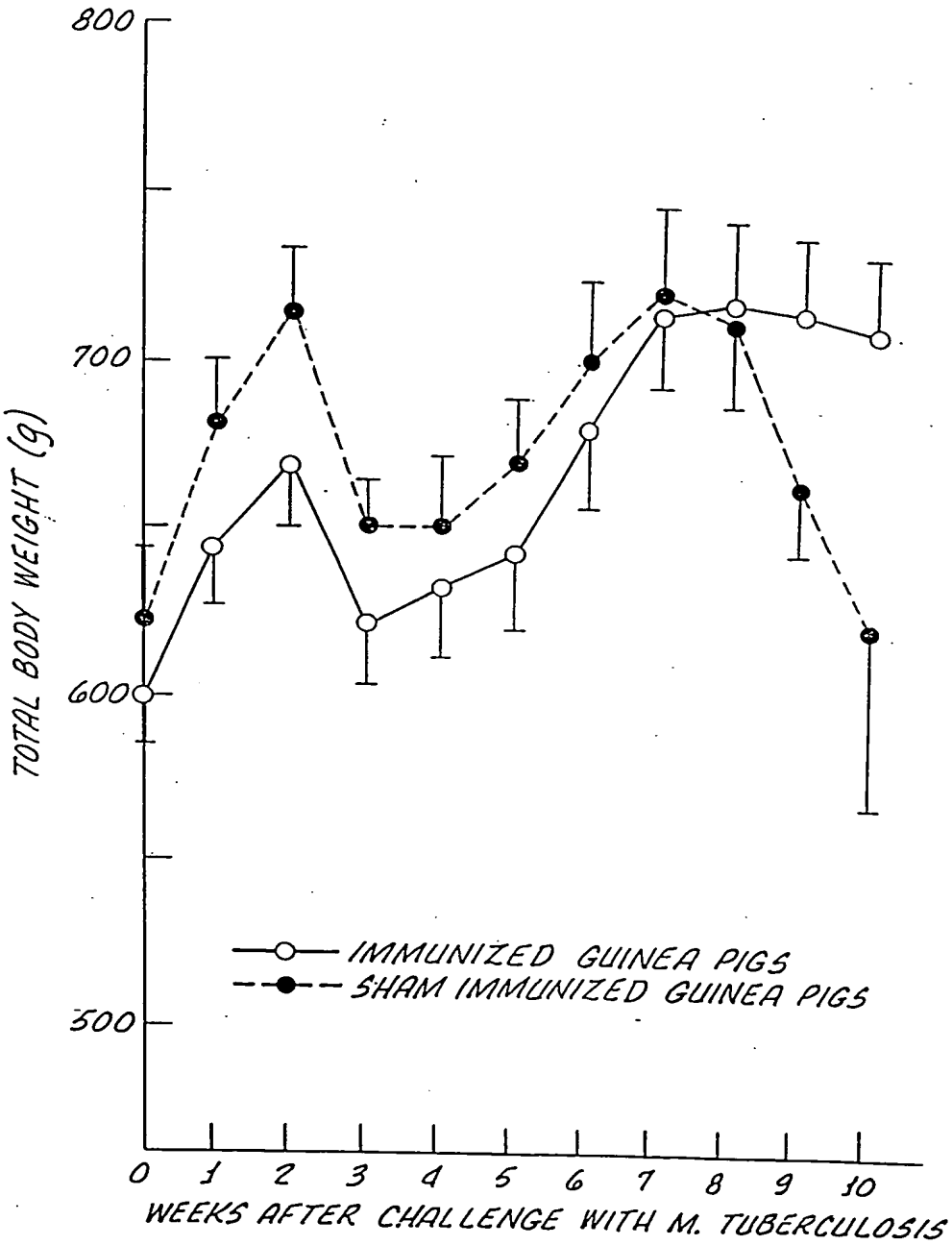

800
700
600
500
TOTAL BODY WEIGHT (g)
IMMUNIZED GUINEA PIGS
SHAM IMMUNIZED GUINEA PIGS
0 1 2 3 4 5 6 7 8 9 10
WEEKS AFTER CHALLENGE WITH M. TUBERCULOSIS

FIG. 9.

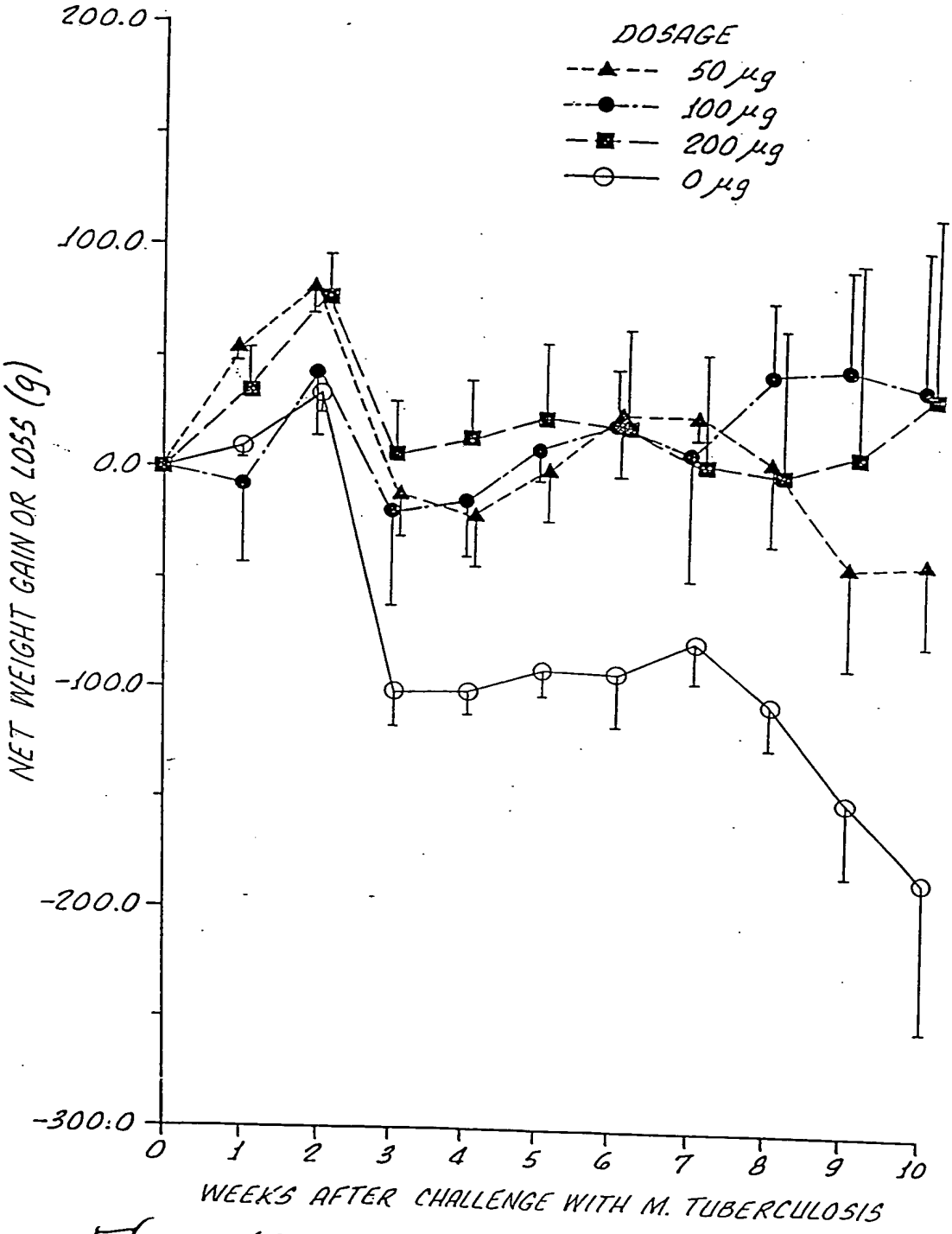

200.0
100.0
0.0
-100.0
-200.0
-300.0
NET WEIGHT GAIN OR LOSS (g)
DOSAGE
50 µg
100 µg
200 µg
0 µg
0
1
2
3
4
5
6
7
8
9
10
WEEKS AFTER CHALLENGE WITH M. TUBERCULOSIS

FIG. 10.

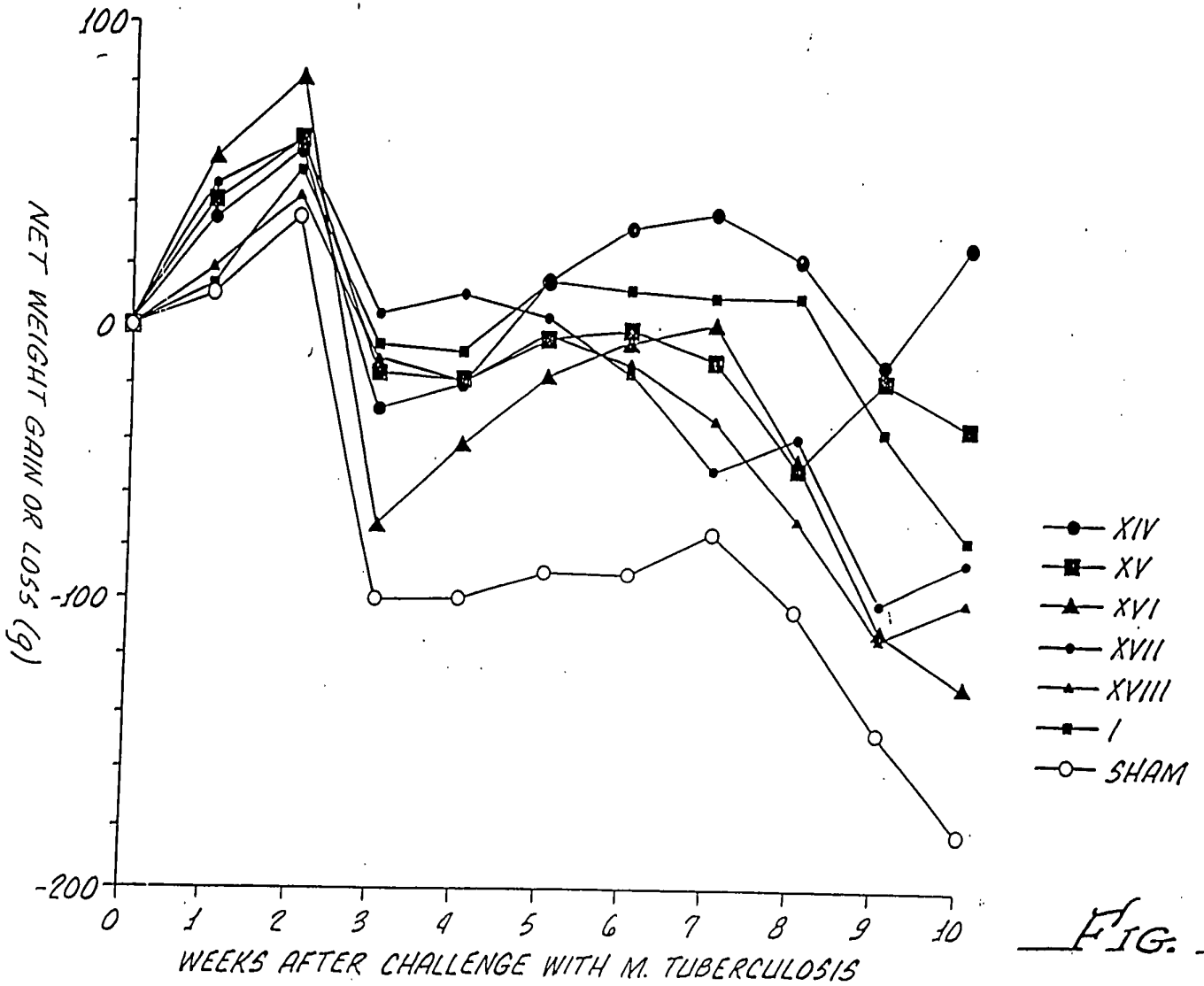

100
0
-100
-200
NET WEIGHT GAIN OR LOSS (g)
0
1
2
3
4
5
6
7
8
9
10
WEEKS AFTER CHALLENGE WITH M. TUBERCULOSIS
XIV
XV
XVI
XVII
XVIII
I
SHAM

FIG. 11.

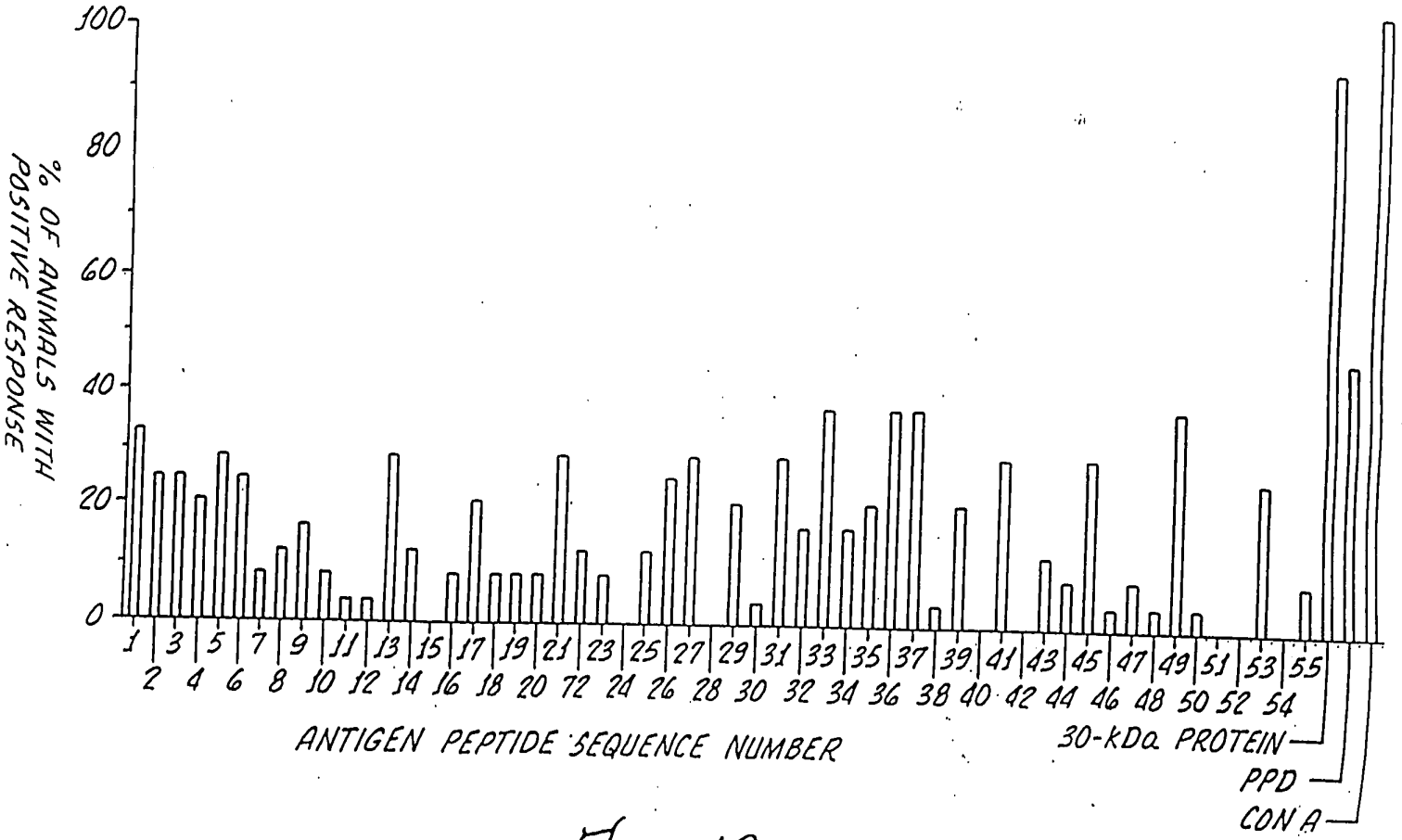

100
80
60
40
20
0
% OF ANIMALS WITH POSITIVE RESPONSE
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 72 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55
ANTIGEN PEPTIDE SEQUENCE NUMBER
30-kDa PROTEIN
PPD
CON A

FIG. 12a.

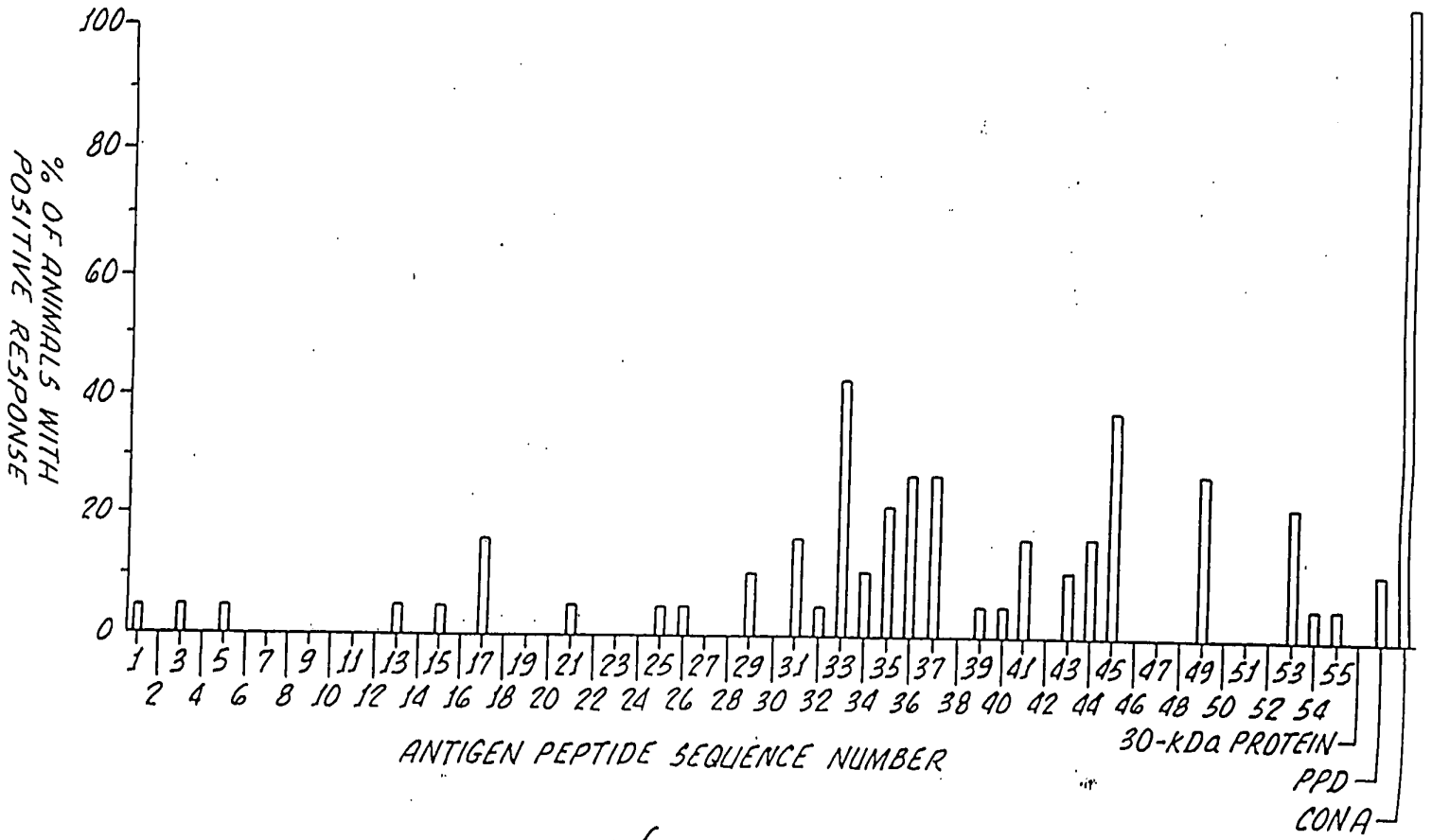
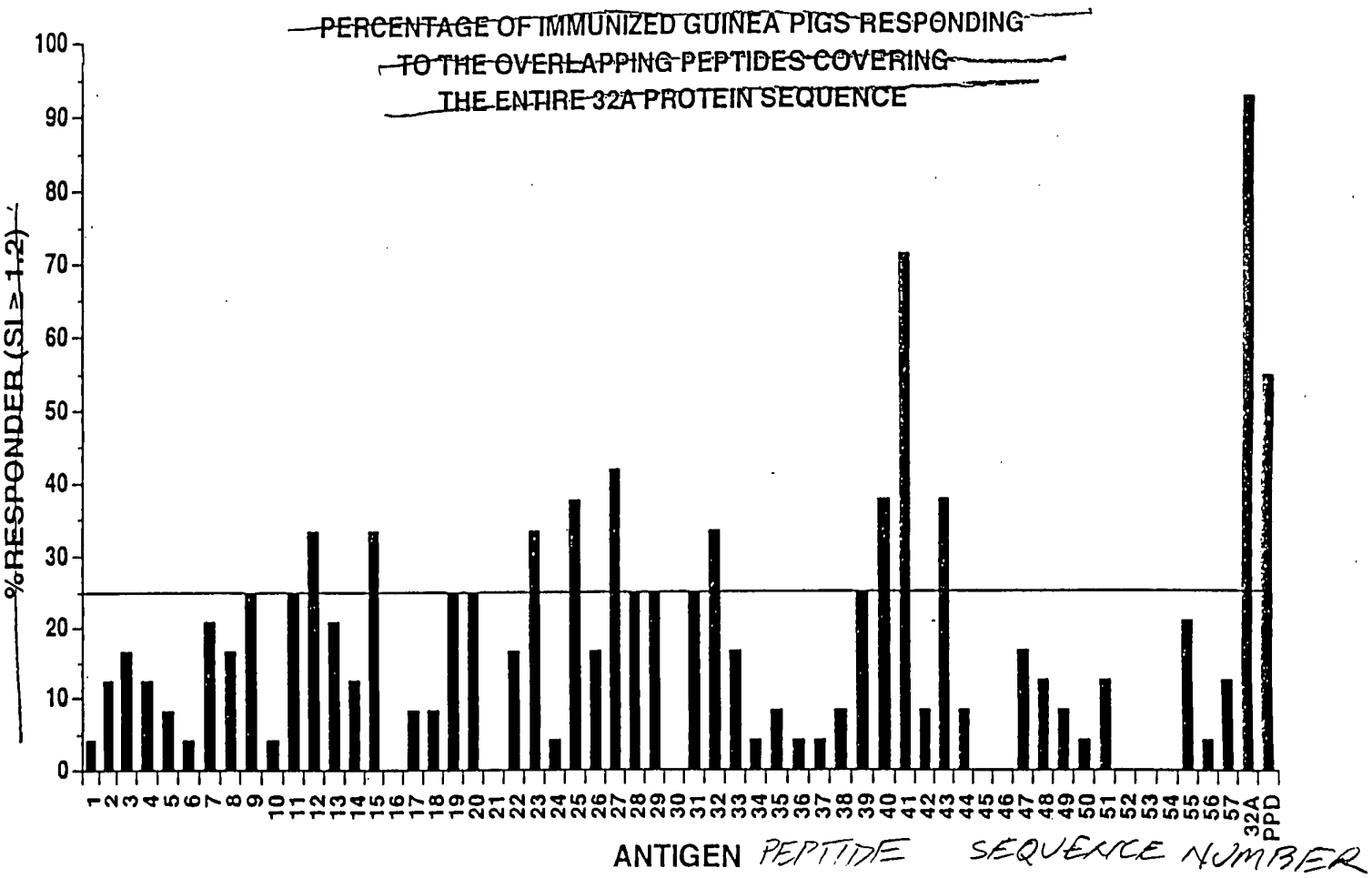

% OF ANIMALS WITH POSITIVE RESPONSE
100
80
60
40
20
0
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52 54
ANTIGEN PEPTIDE SEQUENCE NUMBER
30-KDa PROTEIN
PPD
CONA

FIG. 12b.



No of animals with positive response

FIG 13

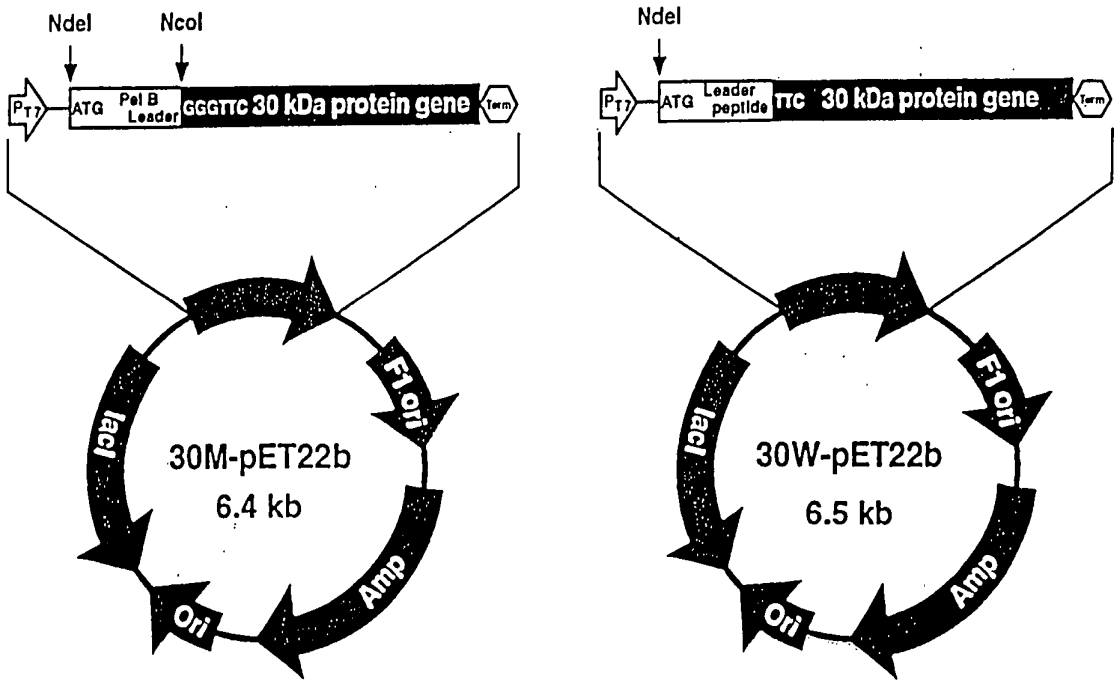

NdeI
NcoI
PT7
ATG
PelB Leader
GGGTTC 30 kDa protein gene
Term
lacI
F1 ori
30M-pET22b
6.4 kb
Amp
Ori
NdeI
PT7
ATG
Leader peptide
TTC 30 kDa protein gene
Term
lacI
F1 ori
30W-pET22b
6.5 kb
Amp
Ori

FIG. 14

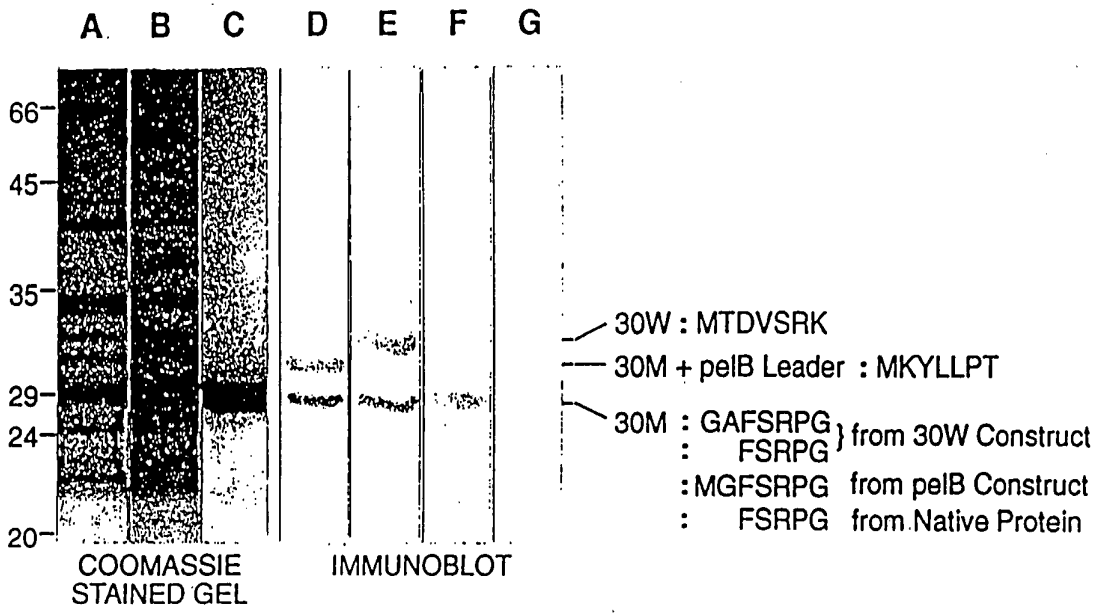

A B C D E F G
66
45
35
29
24
20
COOMASSIE STAINED GEL
IMMUNOBLOT
30W : MTDVSRK
30M + pelB Leader : MKYLLPT
30M : GAFSRPG
: FSRPG
} from 30W Construct
: MGFSRPG from pelB Construct
: FSRPG from Native Protein

FIG. 15.

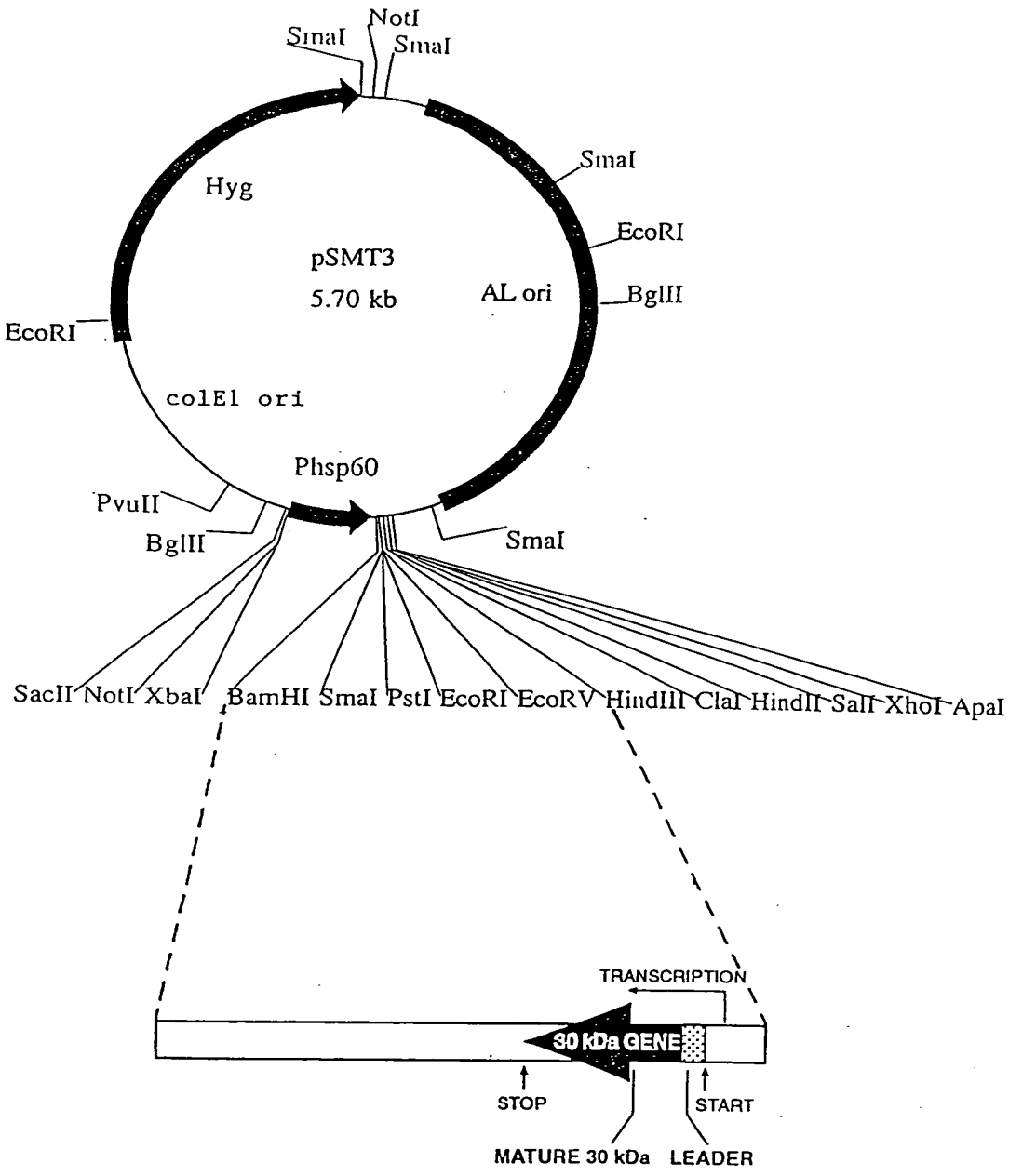

NotI
SmaI
SmaI
SmaI
EcoRI
BglII
EcoRI
Hyg
pSMT3
5.70 kb
AL ori
colE1 ori
Phsp60
PvuII
BglII
SmaI
SacII NotI XbaI BamHI SmaI PstI EcoRI EcoRV HindIII ClaI HindII SalI XhoI ApaI
TRANSCRIPTION
30 kDa GENE
STOP
START
MATURE 30 kDa
LEADER

FIG. 16.

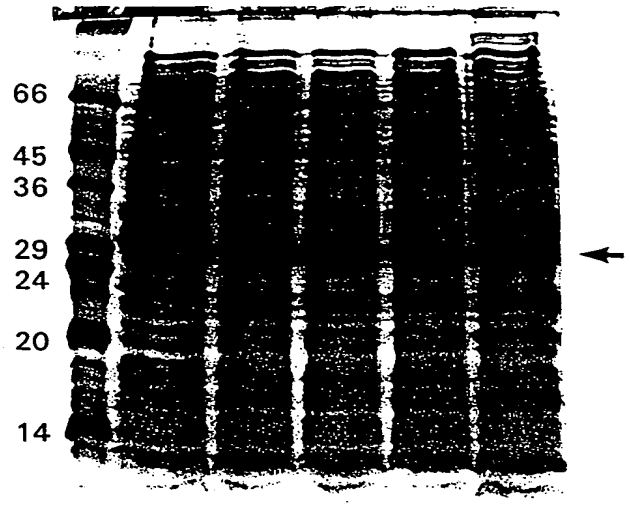


FIG. 17.

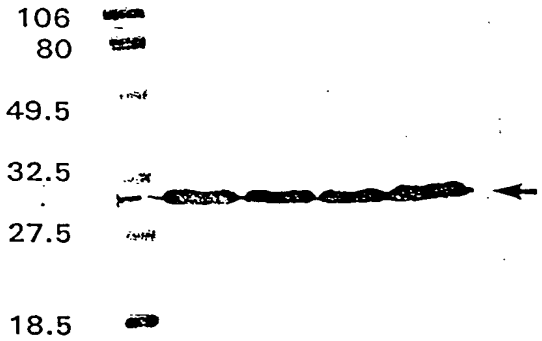
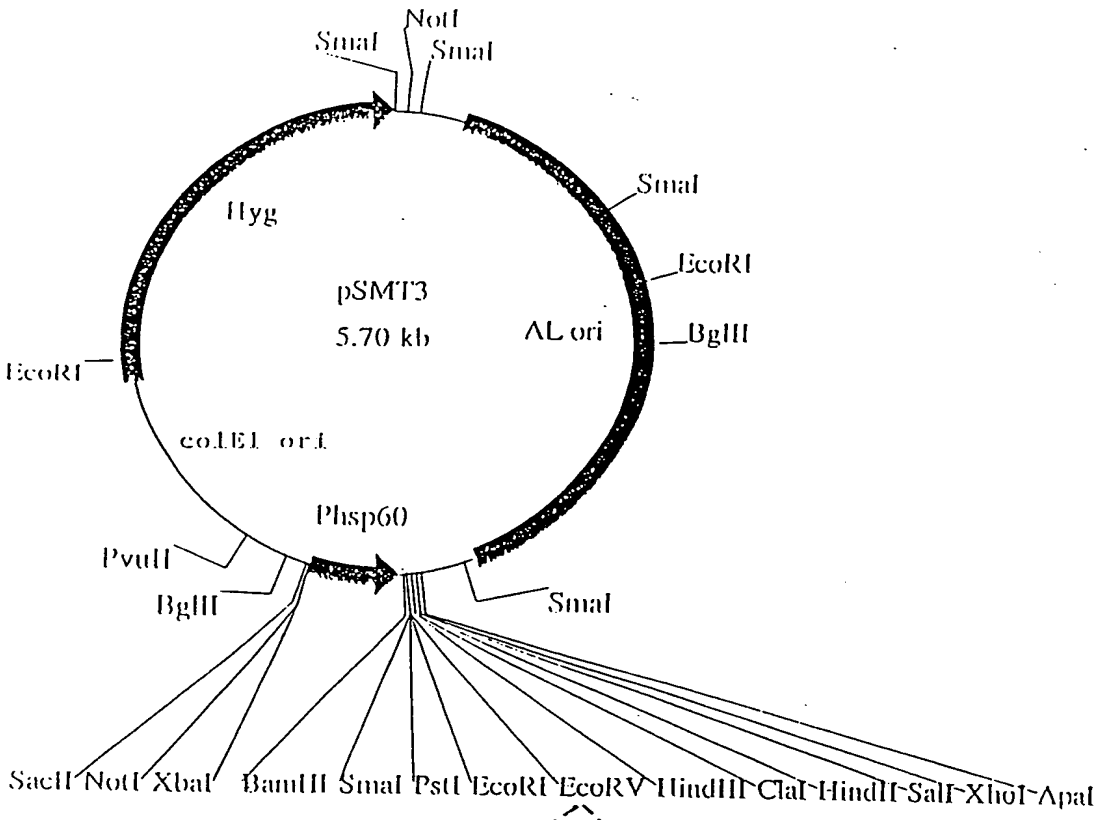
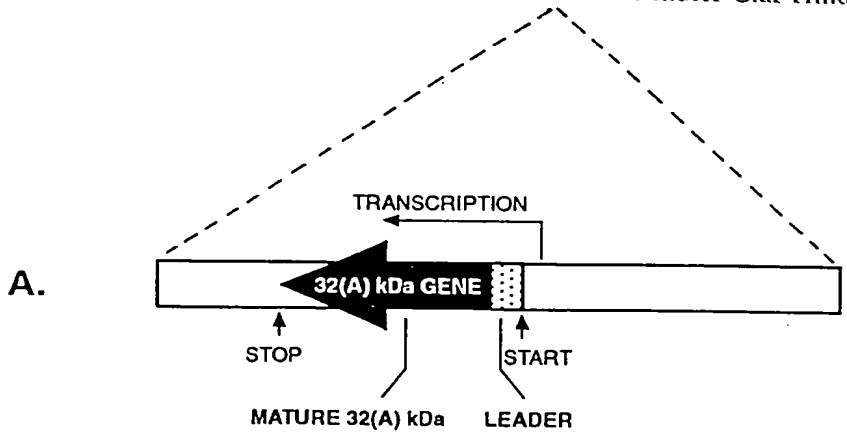


FIG. 18.


NotI
SmaI
SmaI
SmaI
Hyg
EcoRI
pSMT3
5.70 kb
AL ori
BglII
EcoRI
coIE1 ori
Phsp60
PvuII
BglII
SmaI
SacII NotI XbaI BamHI SmaI PstI EcoRI EcoRV HindIII ClaI HindII SalI XhoI ApaI


A.
TRANSCRIPTION
32(A) kDa GENE
STOP
START
MATURE 32(A) kDa
LEADER

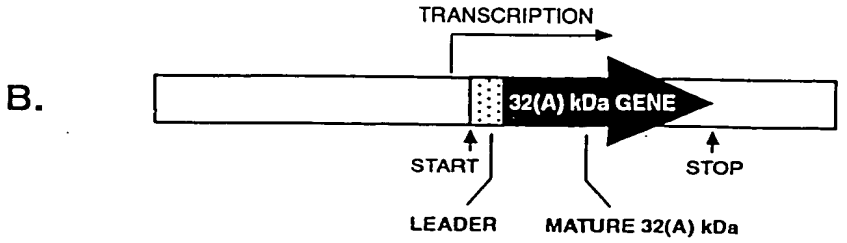

B.
TRANSCRIPTION
32(A) kDa GENE
START
STOP
LEADER
MATURE 32(A) kDa

FIG. 19

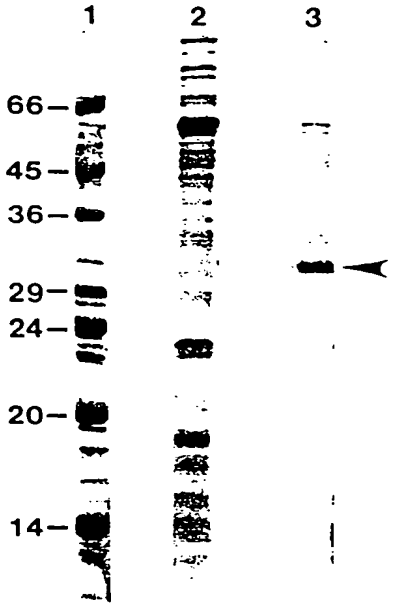


FIG. 20

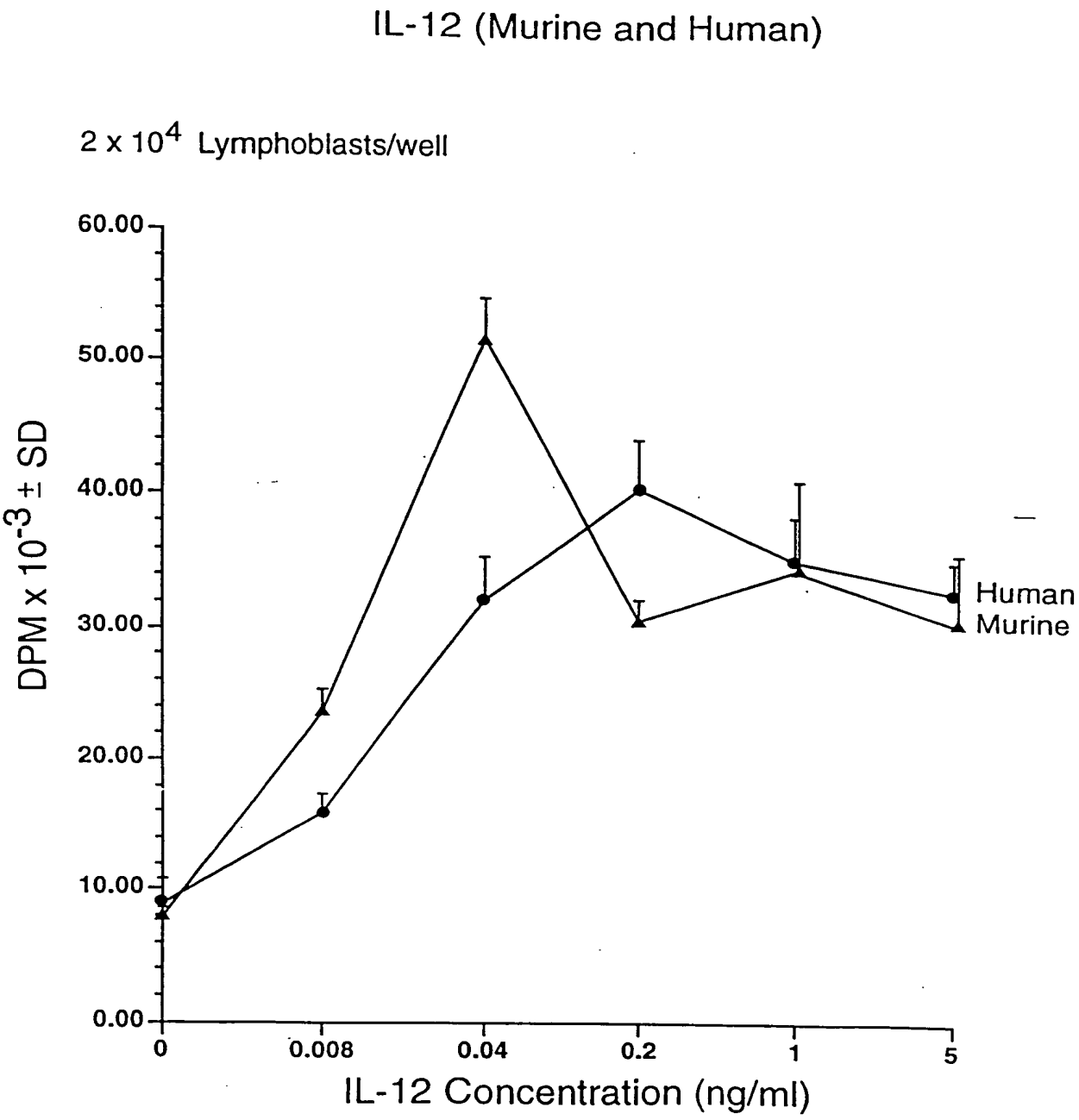

IL-12 (Murine and Human)
2 x 10^4 Lymphoblasts/well
DPM x 10^-3 ± SD
60.00
50.00
40.00
30.00
20.00
10.00
0.00
0
0.008
0.04
0.2
1
5
IL-12 Concentration (ng/ml)
Human
Murine

FIG. 21

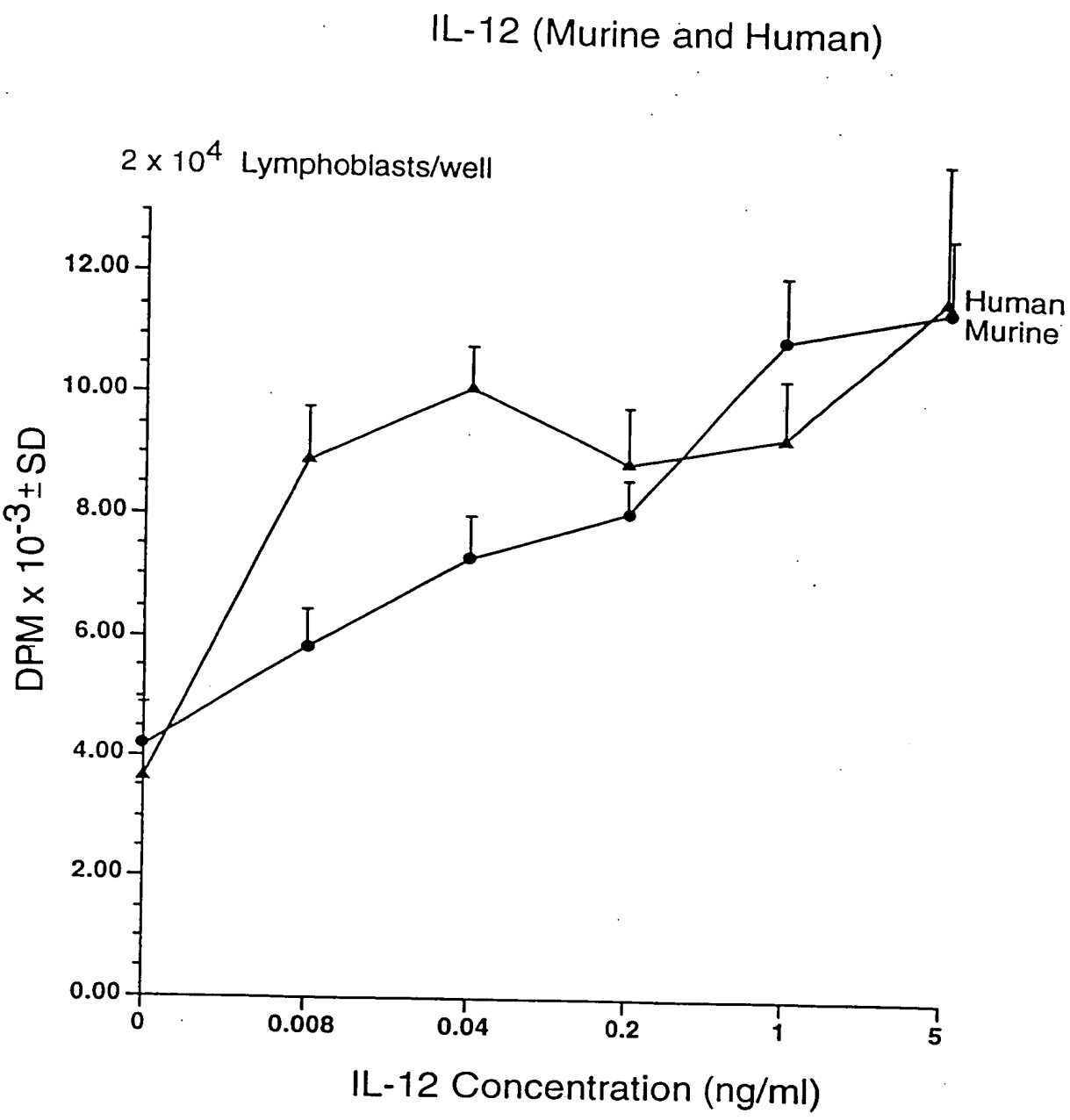

IL-12 (Murine and Human)
2 x 10^4 Lymphoblasts/well
DPM x 10^-3 ± SD
12.00
10.00
8.00
6.00
4.00
2.00
0.00
0
0.008
0.04
0.2
1
5
IL-12 Concentration (ng/ml)
Human
Murine

FIG. 22

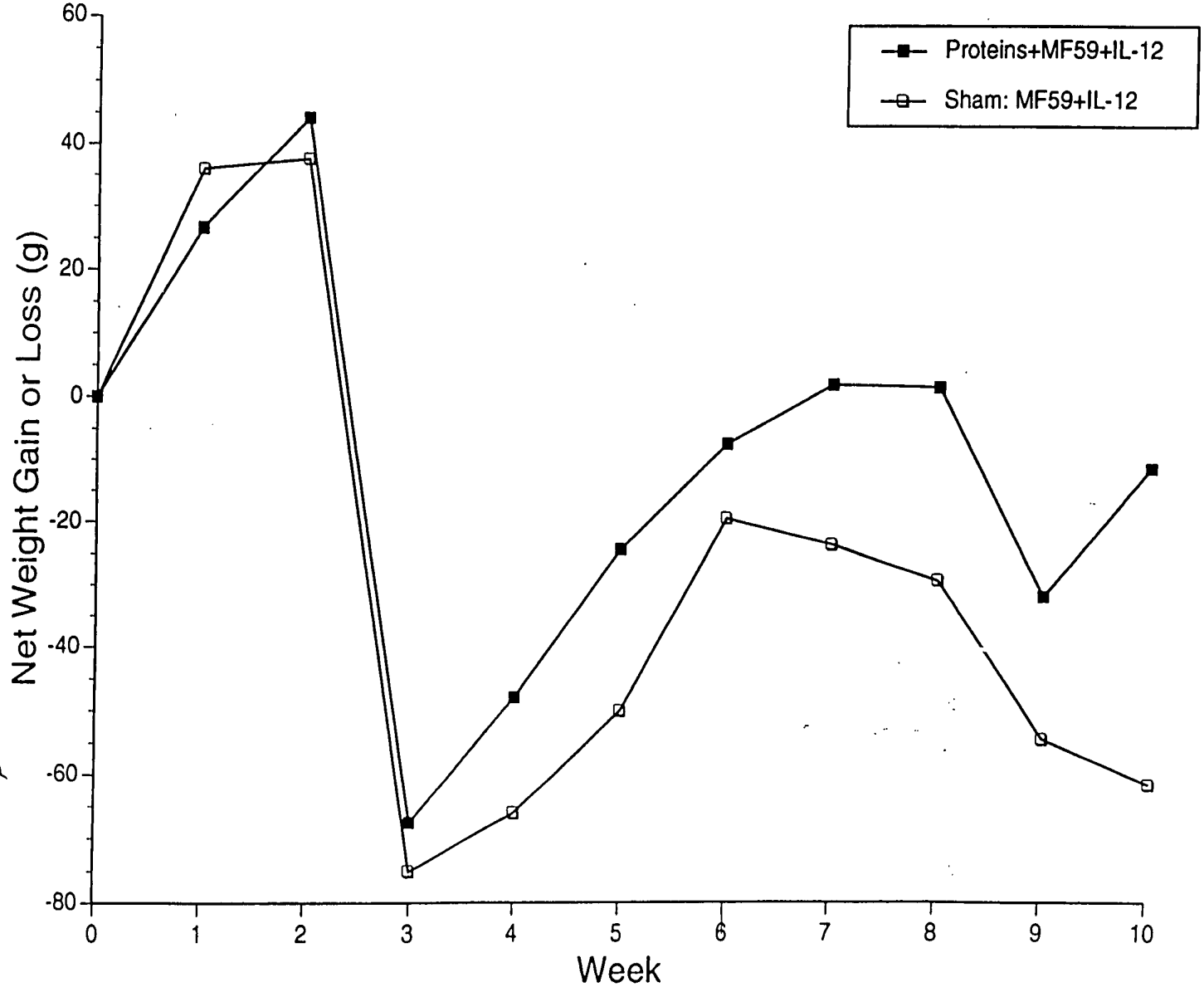

Proteins+MF59+IL-12
Sham: MF59+IL-12
Net Weight Gain or Loss (g)
Week
60
40
20
0
-20
-40
-60
-80
0
1
2
3
4
5
6
7
8
9
10

FIG. 23

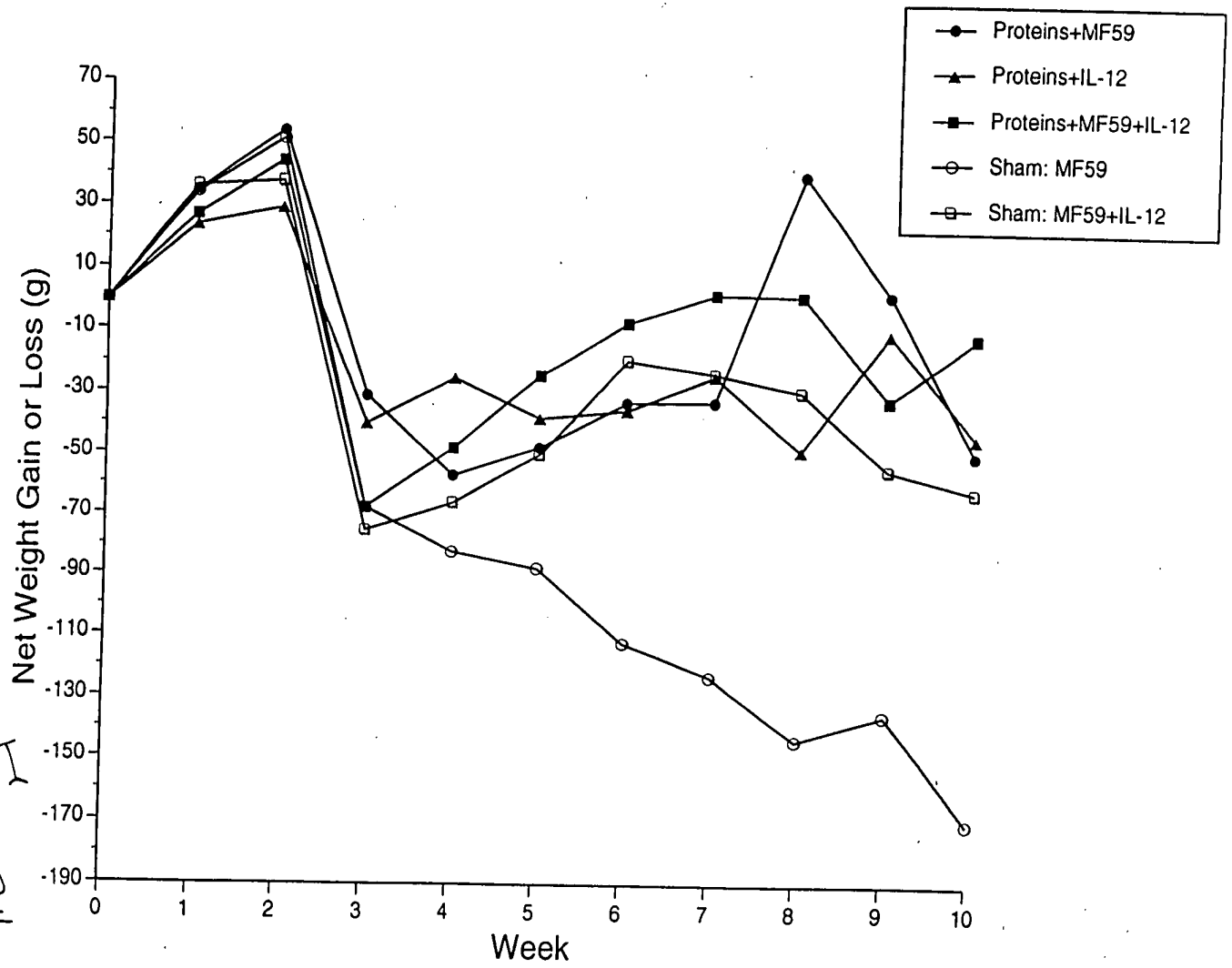

Proteins+MF59
Proteins+IL-12
Proteins+MF59+IL-12
Sham: MF59
Sham: MF59+IL-12
Net Weight Gain or Loss (g)
70
50
30
10
-10
-30
-50
-70
-90
-110
-130
-150
-170
-190
0
1
2
3
4
5
6
7
8
9
10
Week

FIG. 24

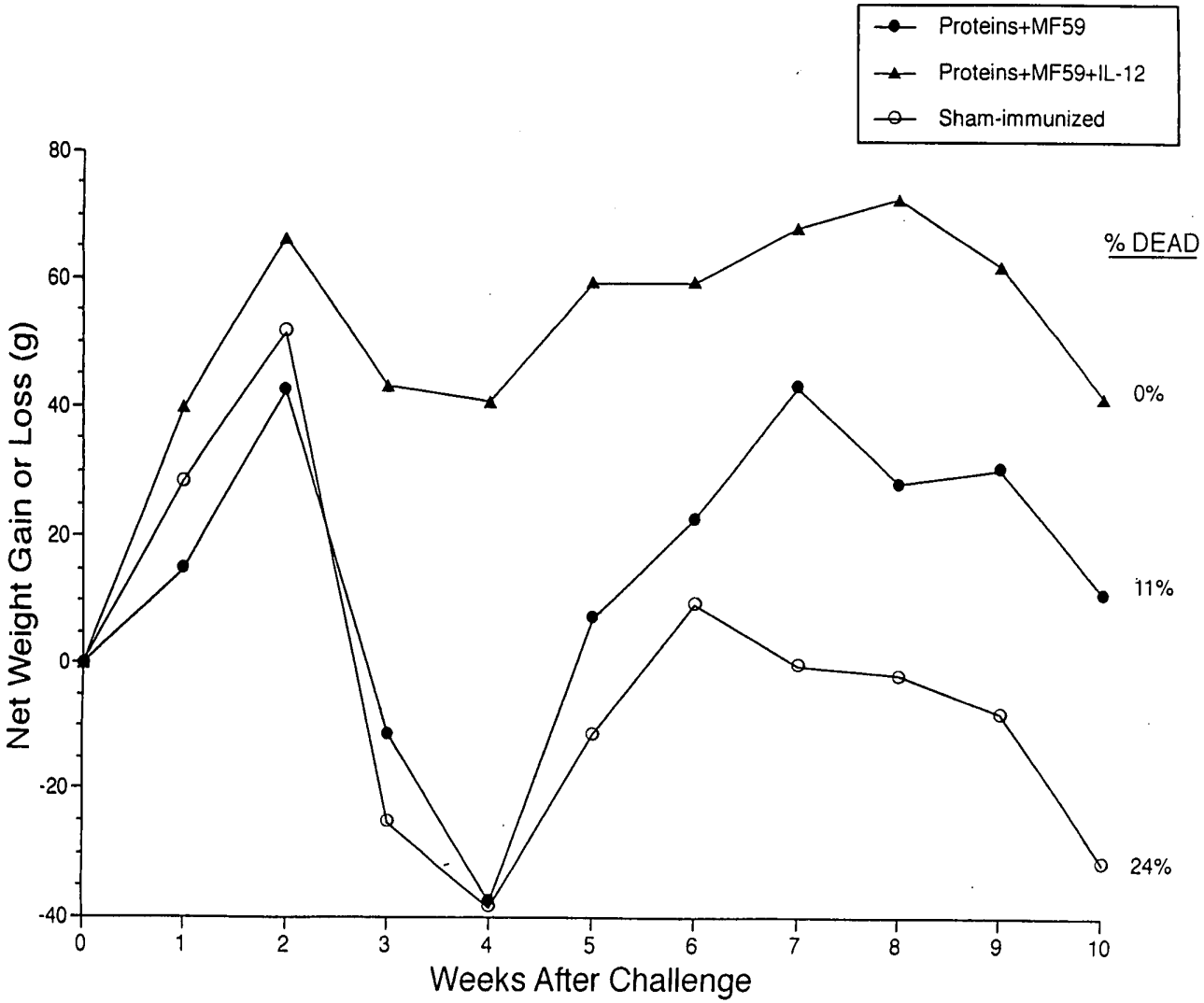

Proteins+MF59
Proteins+MF59+IL-12
Sham-immunized
Net Weight Gain or Loss (g)
80
60
40
20
0
-20
-40
0
1
2
3
4
5
6
7
8
9
10
Weeks After Challenge
% DEAD
0%
11%
24%

Fig. 25